2016

Sponges in Captivity: Creating a Closed System in Which Sponges Can Thrive

Andrea L Schmidt
THE FLORIDA STATE UNIVERSITY
COLLEGE OF ARTS AND SCIENCES

SPONGES IN CAPTIVITY: CREATING A CLOSED SYSTEM IN WHICH SPONGES CAN THRIVE

By

ANDREA LEE SCHMIDT

A Thesis submitted to the
Department of Biological Sciences
in partial fulfillment of the requirements for graduation with
Honors in the Major

Degree Awarded:
Spring 2016
The members of the Defense Committee approve the thesis of Andrea Lee Schmidt defended on April 8th, 2016.

______________________________
Dr. Janie L. Wulff
Thesis Director

______________________________
Dr. William C. Parker
Outside Committee Member

______________________________
Dr. Joseph Travis
Committee Member
Acknowledgements

THANK YOU...To Dr. Janie L. Wulff for devoting the time to help me become a better scientist. Thank you allowing me to be independent with the go ahead of this project and encouraging me to think creatively—I would not have made it to the start (let alone the end!) without you. I have grown as a scientist and as a person as a result of being in your lab and I hope to make you proud as I go out into the real world! To my committee members Dr. Joe Travis and Dr. Bill Parker. Thank you Dr. Travis for being a great sounding board for ideas, even at the very start of this project. Thank you to Dr. Parker for your patience with the arrival of this thesis paper and for speaking with me about forams. To Dr. Greg Hoffman for offering the space near his boat slip and for being constantly curious about the goings on of this project. We’ll find that orange sponge someday. To Annie for being the sweetest dog ever and making lying on a dock full of splinters enjoyable. Thank you for not eating my sponges. To Kate Hill for welcoming me as her field assistant when we were both new to FSU. I am so happy I’ve been able to work with you the last three years. Thank you for always being encouraging and quick to help with my project even when it meant I could no longer help with yours! To Katie Kaiser for giving excellent feedback, for being able to identify sponges like a boss and for helping me with field work. Thanks for driving all over, singing in the car with me and helping me relearn statistics to help this project exist.

To Samuel Bedgood for his incredibly helpful tips and tricks as a skilled aquarist and builder, and for being a sounding board for all of the crazy ideas. Thanks for all of the filter socks and for being just as excited as me about this project. To Will Ballentine for being my biology rock, cheerleader, partner in crime and occasional chauffer. I couldn’t have made it through this year without you, I’m excited to see what our futures hold—as real scientists. I think by now we’ve both earned the title. To Aaron James Bennett for keeping me sane and for being so patient with all of the ups and downs of this project. Thank you for always offering innovative ideas; you are ever the engineer. I hope I can help you with all of your anthropology work as much as you’ve helped me with my biology. Thanks for driving me all over the Panhandle for this project. Thanks for being willing to put dates on hold for the sake of science! To Samantha Levell without whom I would not have passed my classes this semester, nor would I have felt so calm about my own project. Your poise and intelligence are grounding and inspiring. Thank you for feeding me. To Luke Maeder, Josh Buckner, Lynee Tieu, Garret Murray, Olivia Houghton, Marcella Cimarelli, Sarah Cody and Yooyeon Jung for allowing several “beach days” to turn into me making you all field assistants in some way. To Josh Logan for his encouragement, wit and for liking me just the way I am—with sponges. To my family for being encouraging and pragmatic. To Immeke Schmidt for being my shield maiden and all of her help with preparing my defense with words and with battle armor.

And last, but certainly not least, to Gulf Specimen Marine Lab for sharing their living dock with my project in its beginning stages. The sponges I collected were full of other organisms as well, a testament to your mission of being full of marine life. Finally, to the Edith and Lamar Trott Scholarship and The Bess H. Ward Honors in the Major Grant without both of which this project would have been impossible.
# Table of Contents

Abstract.................................................................................................................................................. 5

I. Keeping Sponges in the Lab
   1. Introduction.................................................................................................................................. 6
   2. Methods......................................................................................................................................... 10
   3. Results.......................................................................................................................................... 17
   4. Discussion..................................................................................................................................... 21

II. Some Natural History about *Halichondria corrugata*
   1. Introduction................................................................................................................................. 25
   2. Methods....................................................................................................................................... 26
   3. Results.......................................................................................................................................... 28
   4. Discussion..................................................................................................................................... 30

III. Conclusions...................................................................................................................................... 32

Bibliography........................................................................................................................................... 33
Abstract

Sponges play key ecological roles, from cementing coral rubble in place for coral larvae settlement to acting as a food source for a myriad of organisms, and are a worthy organism for study. Although it makes more sense to perform most ecological experiments in the field, some experiments require a tank set up. The use of tanks can help determine which aspects of sponge biology drive ecological patterns; feeding trials, predation experiments, climate change studies and observations of cellular behavior are all questions better answered in a completely controlled environment. Within both the scientific and aquarist communities it is considered difficult to maintain sponges in a tank setting. In this Honors in the Major project I sought to understand which aspects of sponge biology are important for keeping sponges in captivity. My goal was to keep sponges alive in captivity for the sake of long-term experimentation. There are descriptions in the literature of the utilization of closed systems in laboratory settings, but in many studies, sponges were only kept alive for several weeks before they perished. Through the course of this project I have maintained live *Halichondria corrugata* samples for 13 weeks (at the time of publication). Sponge health has been assessed by comparing the growth of sponges in a lab to sponges at a dock site. I also replicated this tank system in a scaled down version to determine if the volume of water in a system matters for sponge growth. Observations in this closed system have yielded new observations about the species *Halichondria corrugata*, including the possible presence of photosynthetic microbial symbionts. The information gleaned from the many components of this project serves as a strong beginning for keeping sponges for extended periods of time in aquaria as well as more directions to pursue in studying the sponge, *Halichondria corrugata.*
Chapter 1: Keeping Sponges in the Lab

Introduction

Within both the scientific and aquarist communities it is considered difficult to maintain sponges in a tank setting. My project aims to set up a tank system that allows sponges to thrive in a lab environment. Sponges are simple filter feeders, but their filtering ability is incredibly powerful (Reiswig 1971, Strimaitis 2012). To maintain sponges in the lab without negatively affecting their health a specialized tank with water flow is required to support their large filtering capacity and their diet of small prokaryotes and picoplankton. Closed systems have been utilized in laboratory settings, but many were only able to keep sponges alive or at less than ideal health for experimentation for several weeks (Osinga et al. 2001, Duckworth et al. 2012, Duckworth et al. 2004). I argue that sponges which grow in the lab in a way that is similar to how sponges grow in the wild is ideal for experimentation. If similar growth in both settings is attained it would minimize any biases in the results that would arise from sponges being grown in a lab setting and would provide a setting for ecologically relevant experiments.

Importance for keeping sponges in a lab

Laboratory experiments are necessary to isolate specific variables to test. They can also serve to augment a variety of different field experiments. Aquarium systems that keep sponges for extended periods of time can be used for a myriad of experiments, but not at the exclusion of long-term fieldwork. Much of the great work that has been done for sponge ecology has followed individuals in the field through time and how sponges interact with the organisms around them, playing key ecosystem roles (Wulff and Buss 1979, Wulff 1984, Wulff 1994, Wulff 2002).

To test for local adaptation, sponges can be grown in a common garden tank to remove previous environmental effects before performing a reciprocal transplant or exposing them to a particular treatment. Molecular work, which could analyze cellular and genetic responses in sponge cells to stochastic or climactic events, could only be performed in the lab (Barshis et al. 2013). Tissue samples could be taken at different time points after the tank settings were changed to check the sponges’ cellualar response through time. Tank experiments can be used to determine resilience to particular environmental conditions that are logistically impossible to control in the field or could be potentially harmful to other organisms. Climate change studies often manipulate tank conditions over a short period of time (Grottoli et al. 2006, Barshis et al. 2013), but this can confound the response to the treatment with the response to the tank condition. In an aquarium set-up where sponges can be maintained over a long time period, environmental conditions can be manipulated after the sponges have acclimated to the tank. Feeding trials in the field require transplanting sponges onto a movable substrate and placing that substrate in a sealed container. Then one must analyze the microorganisms in the water before and after a sponge filter feeds (Strimaitis 2012). Performing this experiment in a tank would streamline the process for testing feeding specificity and preference in sponges (Osinga et al. 2001). Predation experiments, which require a variety of live sponges on hand, could be performed more rapidly and perhaps with more trials by eliminating a trip to the field every time the researcher requires a new set of sponges.
Several studies have been conducted with sponges in laboratory settings that look at specific aspects of sponge composition, metabolite synthesis and response to environmental conditions. Information on tank size, amount of water flow, amount and type of food the sponges were given and so forth, all guided the tank design for this project. The chart below lists specific studies, the experimental goal of the study, the tank design used in the study and how long sponges were kept in the system. Some studies were more successful than others in terms of the length of sponge cultivation. Most studies had different goals than this current study, which was to create a system that will augment ecological experiments. The successes of the studies in accomplishing their goals with the listed tank design were all taken into account with the design of the tank system for this project.

<table>
<thead>
<tr>
<th>Paper</th>
<th>Goal</th>
<th>Tank Design</th>
<th>Experiment and results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duckworth et al. 2003</td>
<td>To determine the feeding regime that will maximize the yield of the secondary metabolite, Stevensine via culturing the sponge, <em>Axinella corrugata</em>.</td>
<td>12 tanks of each of 15L UV light filtered water, 2 air stones in tank, 8 hour light/16 hour dark cycle, temp: 25˚C, salinity: 35ppt, pH: 8.1. Three sponge pieces per tank, untethered on a mesh support, water changes every two weeks to remove excess waste in water.</td>
<td>Fed multispecific diet at varying concentrations, fed five times per week for eight weeks with 9 explants per feeding regimen. Food materials included, microalga <em>Isochrysis galbana</em>, the yeast <em>Saccharomyces cerevisiae</em>. All explants survived, none reattached to substrate in tank.</td>
</tr>
<tr>
<td>Duckworth et al. 2004</td>
<td>To determine the importance of yeast and other large celled bacteria in feeding and nutrition of <em>Halichondria melanadocia</em></td>
<td>Three 80L aquaria with mechanical and biological filters with about 26 sponges each to allow sponges to heal. Then 18 tanks of 15L each filled with UV filtered water, changed five times per week for six weeks, Two air stones per tank.</td>
<td>Fed food in two different size classes including <em>Marinococcus halophilus</em>, <em>Vibrio alginolyticus</em>, <em>Escherichia coli</em>, <em>Bacillus subtilis</em>, <em>Isochrysis galbana</em> and yeast <em>Saccharomyces cerevisiae</em>. Greater</td>
</tr>
<tr>
<td>Study</td>
<td>Purpose</td>
<td>Methods</td>
<td>Results</td>
</tr>
<tr>
<td>------------------</td>
<td>-------------------------------------------------------------------------</td>
<td>-------------------------------------------------------------------------</td>
<td>-------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Duckworth et al. 2012</td>
<td>To determine how increased temperature and decreased pH affected the growth rates and secondary metabolite synthesis in several species of sponges: <em>Aiolochroia crassa</em>, <em>Aplysina cauliformis</em>, <em>Aplysina fistularis</em>, <em>Ectyoplasia ferox</em>, <em>Iotrochota birotulata</em> and <em>Smenospongia conulosa</em></td>
<td>Three 52 L tanks for each of four treatments, used seawater flow through at 34ppt, five explants per tank with randomization of which species was in which tank. Experiment lasted for 24 days.</td>
<td>pH increased with added CO$_2$ to incoming water, temperature increased using water heaters. Growth was unaffected by pH or temperature, survival was 78% for <em>Aiolochroia crassa</em> and 82% for <em>Smenospongia conulosa</em>. 100% survival for other species.</td>
</tr>
<tr>
<td>Issacs et al. 2009</td>
<td>To determine differences in internal bacterial communities in <em>Clathria prolifera</em> in captivity and in the wild</td>
<td>Five individuals initially kept in plastic bags to acclimate then, into tanks (size not specified). Color loss after one week, after two months they transferred to set up with same construction as in Mohamed et al. 2008. (They did not mention a protein skimmer, which is seen in Mohamed et al. 2008). Fed three times weekly, then once weekly. Fed either Isochrysis, <em>Nanochloropsis</em> or 1:1 ratio of the two. Tested health by reaggregation rates by squeezing sponges through 50µm mesh. Experiment ran for 24 weeks, with sponges remaining “viable” as defined by reaggregation rates and despite loss of vibrant color.</td>
<td>Fed three times weekly, then once weekly. Fed either Isochrysis, <em>Nanochloropsis</em> or 1:1 ratio of the two. Tested health by reaggregation rates by squeezing sponges through 50µm mesh. Experiment ran for 24 weeks, with sponges remaining “viable” as defined by reaggregation rates and despite loss of vibrant color.</td>
</tr>
<tr>
<td>Mohamed et al. 2008</td>
<td>To determine differences in internal bacterial communities in <em>Clathria prolifera</em> in captivity and in the wild</td>
<td>Flow through and recirculating system. Recirculating system with two sponges.</td>
<td>No growth was observed in recirculating tank sponge, color</td>
</tr>
<tr>
<td></td>
<td>Myclae laxissima in captivity and in the wild</td>
<td>800L system with four 80 L tanks and two 160 liter tanks. Flow into sump tank controlled with 100µm filter, sump water into bio filter and algal turf scrubber with protein skimmer. Lights on daily cycle.</td>
<td>change from black to grey, One sponge from recirculating system was compared to one sponge collected from the wild. Notes difficulty of getting sponges to grow in a completely closed system.</td>
</tr>
<tr>
<td>------------</td>
<td>---------------------------------------------</td>
<td>------------------------------------------------------------------------------------------------</td>
<td>-------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Osinga et al. 2001</td>
<td>To determine filtration rate and appropriate feeding regime in the sponge Psuedosuberites aff. andrewsi</td>
<td>80L tanks with sponges tied to Perspex or plastic mesh, mentions same set up as “described by Osinga et al. (1999c)” but the introduction in that paper lists no methodology for tank set up.</td>
<td>Note that qualitative aspect of feeding is one of the most important for sponge aquaculture, did not succeed in creating a system which exhibited continuous growth. Collected sponges from a zoo (not the wild as in previously mentioned studies) two explants were removed due to an infection. Zoo sponges exhibited continuous growth. Variable growth was seen in lab sponges on various diets.</td>
</tr>
</tbody>
</table>

**Tank Size & Water Flow**

Previous studies have used a variety of closed systems for experimentation on sponges, from a massive 800L flow recirculating system that lasted for one month (Mohamed et al. 2008) to several small 52L tanks with some small amount of flow that kept most sponges from between 24 days to eight weeks (Duckworth et al. 2012, Duckworth et al. 2003) Bubblers near the bottom of the tank are a common component for several studies where the authors argue that lots of bubbles keep food particles in suspension and promote flow in the tank (Duckworth et al. 2004, Duckworth et al. 2005, Duckworth et al. 2012). Osinga
et al. (1999c) point out that oxygen is essential to sponge aquaculture but that exposure to air is damaging to sponges. Additionally, the effectiveness of flow throughout a tank due to bubblers is limited by overall tank size. In larger systems the effects of bubbling will be much weaker than in a small tank.

**Feeding Regimen and Water Quality**

Various algal cultures and various schedules for feeding their sponges have been used for laboratory experiments. For example, feeding sponges a set amount of *Nannochloropsis* sp. every two to three days (Mohamed et al. 2008) or feeding *Isochrysis*, and *Nannochloropsis* daily in different mixtures, together and separately (Issacs et al. 2009). Duckworth et al. (2003) utilized various bacterial strains and the yeast, *Saccharomyces cerevisiae*. Although *S. cerevisiae* does not occur in the wild it was useful for secondary metabolite synthesis (Duckworth et al. 2003). Besides feeding, the bacterial communities within the tanks are important for healthy sponge growth (Mohamed et al. 2008, Issacs et al. 2009). Wall et al. (2012) examined the effect of cyanobacterial blooms on *Spechiospongia vesparium* and found that rates of sponge filtration were lower during algal blooms than during non-algal blooms. Although the experiment took place *in situ* it highlights the importance of maintaining cleanliness in the tank. In a closed system, like a tank, sponges would have a more difficult time filtering through a bloom because there is no ambient water to dissipate a concentrated bloom. While increased feeding can increase sponge growth, overfeeding can lead to sponge death (Duckworth and Pomponi et al. 2005). That being said, completely sterile tanks with UV filtered water, as in Duckworth et al. (2003) are not ecologically relevant as they do not mimic the environment where sponges are normally found.

In an attempt to fill in these gaps of sponge survivability and ecological relevance in closed systems, a new system was constructed. I assessed how to keep sponges alive in a laboratory for an extended period of time and how sponges in a tank grow compared to their counterparts in the field.

**Methods**

**Tank Set-up**

The tank that housed the lab sponges was constructed according to the following specifications (also figs 1a,b and 2). Please note the letters next to the following component descriptions and use them as an aid for figure 2. Given sponges' rapid filter feeding abilities the 227.1 L aquarium system was designed with a seawater reservoir to keep a large volume of water flowing through the system at all times. A 109.7 L glass aquarium tank (A) was attached to a second 109.7L tank (B) with 2.54 cm diameter PVC piping. Gravity pulled water from tank containing sponges (henceforth “sponge tank”) through a strainer (C) then through a hole drilled in the glass, which was secured with a bulkhead (D), into the reservoir, or sump tank (B). The pipe into the sump tank (E) extended along the surface of the water to mitigate splashing and to push the water close enough to the backside of the tank to force it to flow downwards. Water from the sump tank was then pumped back into the sponge tank with a 1892.7L per hour pump (F). The piping leading into the sponge tank (G) also extended along the surface of the water to mitigate splashing and to push the water close enough to the backside of the tank to force it to flow downwards. An aquarium
filter sponge (H) on the pump kept large particulate matter from returning to the sponge tank. A protein skimmer (I) removed small particulates and food waste from the water before it was returned to the sponge tank. The sump tank was seeded with live rock two weeks before the addition of sponges to allow for a beneficial bacterial community to develop (Yuen 2009).

*figs 1a, b.* On the left is the sponge tank. Note the PVC pipe coming over the right side of the tank, this is the inflow from the sump tank. The submerged pipe with the black strainer on top is the outflow to the sump tank. On the right is the sump tank. In the bottom left corner is the pump which moves water from the sump back to the sponge tank. In the middle of the tank, hanging off the back is the protein skimmer. To its right is a lamp which was added for the benefit of the beneficial organisms on the live rock.

**Defense for various aspects of the tank setup**

I utilized stands for the sponges so that they had a substrate upon which to grow. Sponges in the wild, and especially *Halichondria corrugata*, grow attached to a hard object (personal observation). Attaching sponges to substrate has been common practice in transplant studies (Wulff 1997).

I incorporated a protein skimmer into the tank design on the advice of several aquarium enthusiasts and from its use Mohamed et al. (2008). The protein skimmer removed the need to limit how much the sponges can be fed as it removed waste and uneaten food from the water. Issacs et al. (2009) reduced feeding in their tanks to avoid microalgae fouling to negative effect on sponge survival over the course of the experiment. Sponges in the wild feed daily, sponges in this system need to be able to as well in order to maximize the similarities between a tank and a field setting.

Live rock was selected as a way to create a tank environment that better matched that of the field by providing a natural base for beneficial bacteria to cycle through nitrates. Although I was not able to find live rock used in any previous lab study on sponges, coral species grow faster in captivity with the addition of live rock (Yuen 2009). Many members of the salt water aquarium hobby community swear by the use of live rock and some have even noted sponges hitchhiking into systems on live rock and proliferating in tanks (S. Bedgood, personal communication). Any sponges noted on live rock in this experiment were removed before the addition of experimental sponges.
Nannochloropsis sp. was selected as a food source due to its use in several studies that kept sponges alive for longer than a period of a few weeks (Mohamed et al. 2008 and Issacs et al. 2009).
Maintaining Water Quality

Fig. 2. Annotated diagram of tank setup. 
A - Sponge Tank, B - Sump/reservoir tank, C - Strainer, D - Bulkhead, E - Sponge tank outflow, F - Pump, G - Sump, H - Aquarium sponge filter, I - Protein skimmer, J - Grow light.
I maintained a tight salinity range (22-23 parts per thousand (ppt)) with addition of freshwater each day to counteract evaporation from the tank. During school breaks a Tsunami brand automatic top off device was employed to take the place of humans adding water to the tank. I cleaned the protein skimmer collection cup once per week. I performed a partial water change on the system once per month.

**Pilot work**

The first version of this project was started in November 2015 and ended in January 2016. The sponge collection site was located at the Living Dock associated with Gulf Specimen Marine Lab (30.0134.4, 84.2309.2), henceforth “The Gulf Specimen Dock”. The underside of the wooden dock was covered with styrofoam, which was covered in sponges and other fouling organisms. I selected the sponge, *Halichondria corrugata* Diaz, van Soest, Pomponi 1993 for this study because it was especially abundant along the underside of the dock. It was found in large clumps of relatively uninterrupted sponge, with the occasional ascidian or algal mat. Pieces of *H. corrugata* were collected from all around the empty boat slip at Gulf Specimen’s dock.

Individuals of *H. corrugata* were contiguous on the underside of the dock, so individuals were defined as clumps of sponge at least one meter apart (an assumption to control for genetically different individuals). Thirteen sponges with as few visible symbionts as possible were selected (no anemones, algal mats, colonial tunicates, barnacles). A razor blade was used to make small, repetitive nicks in the sponge tissue until the piece came free from the main part of the animal. Sponge samples were cut in a way to preserve as much as the pinacoderm as possible. The sponge sample was then placed in a submerged Tupperware container and transferred into a large clear storage container filled with seawater to avoid air exposure. This piece was cut into two or three pieces depending on the size. The pieces were measured by volumetric approximation (Wulf 2001) and attached to 5.08cm long nubbins of CPVC using beaded cables ties. The CPVC was boiled in salt water and set to cool before being used with the sponges in order to remove any toxins in the CPVC that might leach out. All nubbins were attached to a PVC pipe (2.54cm diameter, 30.48cm long). The PVC pipe was transferred from the storage container to the canal water via the Tupperware to avoid exposure to air and hung with two UV resistant zip ties tied with parachord to a line (13.6 kg strength) that stretched between two pilings on the land facing side of the dock (fig. 3). The pipes were tied so that at least 2 cm of PVC was below the water’s surface (fig. 4). The main line was tied on D-rings surrounding the pilings of the floating dock so that the line moved with the tides.

After one week all nubbins and sponge pieces were collected and brought back to the lab (growth rates were to be compared with data from the literature). The nubbins were attached to CPVC pipes that were attached to CPVC t-joints on stands (fig 5.) These stands were transported back to the lab in a cooler. Temperature and salinity were monitored daily and water chemistry was checked every 10 days. Salinity was checked using a refractometer. An API Saltwater test kit was used to test ammonia (NH₄⁺) levels.

**Sponge Food**

Sponges were fed 60mL of the phytoplankton *Nanochloropsis* sp. daily. A light timer and 13.24 L/hour pump were employed to give the proper dose on a daily basis. The pump/timer apparatus was calibrated by setting the timer to turn on for two minutes daily
and the pump was placed high enough above the tank to slow down food flow so that only 60mL of food were dosed per day. After 48 days the dosage was doubled to 120mL per day. This was to compensate for when the feeding apparatus malfunctioned at some point between days 41 and 47 (FSU’s spring break). A grow light was added directly above the sponge tank at the same time the dosage was doubled (fig 2, J). 800 mL of seawater was mixed down to a salinity of 26ppt with 0.8ml of the f/2 media MicroAlgae Grow ® and left overnight before autoclaving. Seawater was put on cycle 9 for liquids and then promptly doused in ice once being removed from the autoclave to prevent the food media from precipitating out. This was then left overnight to adjust to room temperature. 200mL of algae from the flask containing the highest concentration of algae were poured into each new flask of seawater. A culture was used to feed to sponges when it was a dark emerald green color, indicative of a high concentration of algae.

The first trial ended in a mass mortality event. Over FSU’s winter break period the auto top-off device that was intended to maintain a constant water level and salinity shut off. This then caused the protein skimmer to stop working. A more in depth analysis of this event is covered in the discussion section. After a 50% water change of the system and removal of all dead sponge tissue, five pipes with varying amounts of sponge tissue were left. These remained in the tank during the second trial to see if they would make a come back. In order to avoid tank effects from positioning, the sponge stands were rotated around the tank every other day.

**fig 3.** Pipes are hung on a parachord line strung between D-rings surrounding two pilings so that the pipes position in the water fluctuates with the tides.

**fig 4.** Pipes are hung so that the top of the pipe is at least 2 cm below the water surface.

**fig 5.** The base of a CPVC stand, with prominent t-joint.
Growth Comparison Between Field and Lab Sites

The study and collection site for the main part of this study was located at Shell Point Marina at the end of one of the resident's docks (30.05878, -84.29148), henceforth known as “Shell Point.” Pieces of Halichondria corrugata were collected from the land-side of the dock, the canal-side and the insides of boat slips. The collection methods for H. corrugata are nearly identical to the pilot study except: the sponge individual was cut into four smaller pieces, all with at least two sides covered in pinacoderm and seven individuals were collected in total.

After one week the nubbins and their sponges placed at the first and fourth positions on the pipe were then measured and brought back to the lab; nubbins at the second and third positions were measured and remained in the field. The water at the field site on the collection day was 15°C while the water in the lab was 23°C. The sponges were left in the cooler over night with a bubbler so that the water temperature in the cooler could equalize to the water temperature in the lab tank to avoid shocking the sponges. The sponges were fed a 30mL dose of Nannochloropsis sp. algae overnight. The water in the lab tanks was mixed to a salinity that fit a range between the initial collecting day the next week (the first collecting day had a salinity of 27ppt, one week later was 23ppt so a median salinity would be 25ppt). Temperature and salinity were monitored daily and water chemistry was checked every 10 days. Salinity was checked using a refractometer. An API Saltwater test kit was used to test nitrates, nitrites, ammonia and pH.

Sponge growth was assessed by measuring the sponge volume at regular intervals (every three weeks for the first two months, every week for the last month). Timing of when the sponge reattached was noted. Reattachment was defined as any new sponge tissue on the CPVC nubbin. The outside of the sponge was measured by approximating volumetric shapes, then the volumes of all the individual shapes were be summed to yield total sponge volume (as in Wulff 2001). Sponge coloration, morphology, and tissue appearance were all noted during measurements.

Comparison of total volume of water between two systems

As the main tank still held live sponges two months into the experiment, a smaller tank system was constructed to test if the volume of water in the system had any effect on how the lab sponges grew. This smaller system held 151.5L of water total with a 75.7L sump tank and two 37.8L sponge tanks (fig 6a, b). Since the volume of water was being examined it was not important that there be one 75.7L sponge tank or two 37.8L sponge tanks. This tank was seeded with live rock from the main tank and left for at least a week before the addition of live sponges. A smaller protein skimmer was purchased for this tank and a 1324.9L/ hour pump was utilized. The piping from the sponge tank to the sump tank was vinyl instead of PVC in order to account for the potential fragility of the glass walls in the 37.8L tank. The vinyl was soaked in warm salt water before being used with the tank and should be as biologically inert as the PVC. The piping from the sump tank to the sponge tank splits about 60cm above the pump into the two separate sponge tanks. The pipe leading into the sponge tank extended along the surface of the water to mitigate splashing and to push the water close enough to the backside of the tank to force it to flow downwards.
The sponges for these smaller tanks were collected using the same methods as described in the “growth comparison between sites” section except only four individuals were collected instead of seven. Additionally the sponges were brought immediately into the lab after collection, rather than a week after initial collection at the dock site. The sponges were left in a cooler with a bubbler and 30mL of *Nannochloropsis* sp. overnight to mimic the conditions in which their larger tank counterparts were collected. These sponges were measured weekly by volumetric approximation.

**Statistical Analysis**

The data were not normally distributed therefore a Wilcoxon-Mann-Whitney test was used to see if the total change in size, specific growth rate and pipe position were significantly different. Excel was used to calculate the $R^2$ value for a linear regression of proportional growth compared to initial size. The specific growth of each individual was calculated by subtracting the initial volume from the final volume and dividing the difference by the initial sponge volume.
Results

Pilot Work

There was almost complete mortality (fig 7) of sponges after about one month. Average specific growth during the week these sponges were acclimating to pipes at the Gulf Specimen dock site was -0.094 cm$^3$.

*fig 7.* Mass mortality of sponges during the pilot work. Note the general lack of live tissue and the remaining skeletons.

Growth Comparison Between Field and Lab Sites

The total average volume change through each measurement point shows a gradual increase in sponges at the dock site and a noticeable decline in sponges in the laboratory (fig 8). This does not tell the whole story, however, and must be examined more closely. Comparing specific growth for the first period (1/16 to 2/13) to specific growth for the second period (2/13 to 3/27) shows how little proportional change the dock sponges underwent whereas the lab sponges lost much more volume (fig 9). Specific growth between dock and lab sponges in stage one was not significantly different (Mann-Whitney-U, $U_s=94, p>0.2$). Specific growth between dock and lab sponges in stage two was significantly different (Mann-Whitney-U, $U_s=0, p<0.0005$). Initial sponge piece size is negatively correlated with specific growth for both stages in the dock sponges and during the first stage for the lab sponges (fig 10a-d). All of the dock sponges (n=14) survived up to this point in the experiment. All but one of the lab sponges survived up to this point (n=13). One of the lab sponges was exhibiting necrotic tissue and was removed before it could negatively affect the other sponges in the tank.

*Fig 8.* Average change in volume at each time point. Note dock sponges size increase toward end of period and lab sponges decline starting at 2/13/16. Left of green line is stage 1, right of green line is stage 2.
Fig 9. In the red are the average specific growth rates for dock sponges from 1/16 to 2/13 (this is the relatively stable period seen in figure 8, to the left of the green line) and from 2/13 to 3/27 (this is the period that starts with a decline. In the blue are the specific growth rates for lab sponges over the same period.

Fig 10 a,b,c,d.. Clockwise from the top left corner are the specific growths for individual sponges plotted against their initial size at the beginning of that time stage. $R^2$ values are listed on the graph. A= period from 1/16-2/13 for dock sponges, B= period from 2/13/-3/27 for dock sponges, C= period from 1/16-2/13 for lab sponges. An outlier, denoted by the black dot at (44.92, -0.537) was removed for the calculation of the $R^2$ value. D= period from 2/13-3/27 for lab sponges.
The location on the pipe (top sponge or bottom sponge) does not yield significantly different growth rates in both the lab and at the dock. There is no significant difference in growth between the top and bottom positions on the pipe for both the lab and dock sites (fig 11a, b).

In an attempt to check if genotype influences the growth of sponges at the two sites the average specific growth for both pieces of an individual in both the lab and the dock were plotted against each other for stage 1 and stage 2 of the experiment (fig 12 a, b). $R^2$ value for stage 1 was 0.269 and 0.083 for stage 2.

It took until day 48 to see reattachment of most dock sponges to their CPVC nubbins. Reattachment by most lab sponges occurred by day 28. The salinity at the Shell Point site had a wide range, from 15ppt up to 27ppt. Temperature ranged from 15°C in January to 22.8°C in March. Salinity in the tank ranged from 22-24ppt and the temperature ranged from 22-24°C. Nitrate (NO$_3^-$), nitrite (NO$_2^-$) and ammonia (NH$_4^+$) levels were constantly at 0ppt, pH was constantly at 8.2.
Comparison of total volume of water between two systems

For the tank system with the smaller volume of water reattachment was observed in half the sponges (n=4) by day 10 in both the lab and at the dock. Specific growth for this period averages 0.578 for dock sponges and 0.192 for lab sponges (fig 13) and this growth is not significantly different between the two sites (Mann-Whitney-U, Uₛ = 24, p=0.1). There is no significant relationship (regression slope, p=0.5) between initial size and specific growth in the dock sponges nor in the lab sponges (fig 14 a,b). The salinity in the tank ranged from 22-23ppt and the temperature raged from 23-24°C. Nitrate (NO₃⁻), nitrite (NO₂⁻) and ammonia (NH₄⁺) levels were constantly at 0ppt, pH was constantly at 8.2.

Fig 13. Average specific growth for lab and dock sponges. Error bars represent standard error

Figs 14a,b. Initial size was plotted against specific growth for the 10 day period. On the left is the dock site (R²=0.10395), on the right is the lab (R²= 0.5627).
Discussion

Using this tank set up, live samples of *Halichondria corrugata* have been continually maintained in a tank setting for 11 weeks. This research project has been a series of trials and errors. Although the errors sometimes lead to less than ideal results they can also lead to important discoveries about what parts of the system are essential. This discussion section will address how specific growth differed between the lab and the field, how we know which parts of the system are essential and which parts need improvement and if the volume of water might matter for sponge growth in a tank experiment.

Growth Comparison Between Field and Lab Sites

The results showed that the specific growth rates in sponge individuals between the field and the tank were different which means this system needs to be reassessed in order to create a system that makes sense for ecological experiments. It is possible that the lab sponges had some small amount of growth in the first stage of the experiment (fig 11) because of the constant temperature and salinity in the lab which the field site lacked. There was an immediate decline in the volume of dock sponges where the lab sponges saw an immediate increase in volume (figs 8 and 9) which could also be due to the lab sponges suddenly being immersed in a warmer environment—where the warmer water could have initially served as a seasonal cue. *Ircinia fasciculata*, a symbiont bearing sponge, has been noted to have increased growth rates during the late spring and summer months due to the increase in temperature which occurs during that time (Turon et al. 2013). It could be posited that given that the equation for specific growth this decline could simply be an artefact of statistics but qualitative data show a definite decline in sponge volume as well (fig 15a-d). The following decline in sponge volume in the lab during stage two could also be due to the absence of a light source for the first 48 days of the experiment, which could cause loss of photosymbionts. Photosymbiont density has been found to be coupled with pigmentation (Liacci 1964b). Issacs et al. (2009), the only study I was able to find which kept sponges in captivity longer than this one also noted a decline in tissue coloration in *Clathria prolifera* through the course of the experiment. It is highly likely that *H. corrugata* hosts photosynthetic microbial symbionts (see chapter two) and it might suffer without light that provides energy to them.

Initial sponge piece size was loosely negatively correlated with specific growth for that sponge. During both stage one and stage two at the dock site the $R^2$ values were 0.23 and 0.38 respectively. So not much of the variance can be explained by the arguably negligible effect of initial piece size on specific growth. In the lab tank the $R^2$ values were 0.510 for stage one and 0.033 for stage two. We can see some of the variance in specific growth in the tank set up can be explained by the initial piece size. The negative slope of the correlation makes sense biologically in terms of resource limitation as a larger piece would need more resources to grow more, in the absence of those resources it would have a much lower specific growth. There is virtually no effect of initial piece size on specific growth in the second stage of the experiment which indicates there are other factors driving the specific growth.

We can see the importance of controlling for genotype in this experiment in figures 12a and b. For the stage one graph (fig 12a) we can see some sponges which had a high specific growth at the dock and a low specific growth in the lab. At the same time there is another cluster in the same graph which did poorly in both the lab and at the dock. For the
stage two comparison (fig 12b) all of the points clustered on the negative side of lab specific growth axis, which matches with the decline we saw in overall specific growth for the lab sponges over this time. Despite this clustering there is still a clump of points where genotypes that shrunk in the lab grew at the dock and a cluster of points where sponges shrunk in both the lab and at the dock. Figure 12 shows a wide smattering of points with R^2 values of 0.269 for stage 1 and 0.0833 for stage 2. These R^2 values do not explain the variance in specific growth that we see which indicates there are other variables affecting the growth so there is no discernable signal from genotype. This could mean some genotypes are better suited to being reared in a lab than others but we cannot draw such conclusions from this data set.

Comparison of total volume of water between two systems

The tank, system with the smaller volume of water, at 10 days in, showed a similar average specific growth compared to its large tank counterpart early into the experiment (0.192 and 0.175 respectively). There are similar R^2 values for the relationship between initial volume and specific growth in both the large and small volume tank set ups. Although the linear regression was not significant, similar R^2 values hint at a similar relationship in both systems. Time will tell if this initial growth will lead to a later decline in total volume or if a smaller total volume of water is actually better for keeping sponges. A smaller volume is counterintuitive to a higher chance of success due to its higher susceptibility to stochastic events. For example, if the salinity changes, it will change much more rapidly and noticeably due to the fact that there is less water throughout to buffer the change. At the same time a smaller set up overall would be beneficial for lab logistics in running one or several long term studies. Noting the volume change through time becomes more powerful when coupled with qualitative data about the sponge’s coloration and morphology. This will be explored more fully in the next chapter.

Two sponge individuals were collected and kept in a separate 9.46L tank with a water pump for flow. Several weeks later they were still alive, despite not having a protein skimmer, regular food or a large source of live rock aided water. They were notably thinner than before and had much more fistulose morphology but they were still alive. I would argue that these individuals were not healthy as this change in morphology was not seen at the dock site. So simply adding a pump to a tank and letting the sponges sit might not be best for mitigating tank effects. That being said, the sponge’s mere survival means this simpler method deserves a more in depth look with quantifiable results.

Discussing various aspects of the tank set up

When I returned from winter break I discovered that the water level in the tank was far below the ideal level, the salinity much higher than the constant 23ppt and the protein skimmer off-- it allowed me to see that the protein skimmer is an essential part of the system. Given that sponges in the field site can handle a wide range of salinities (27ppt to 15ppt) the only other missing piece was the protein skimmer, which was off due to the low water level. Over the spring break period (ending at 3/4/16) the feeding pump stopped working so the sponges starved for an unknown period of time. This was noticeable due to the extreme size change before and after the break (see figs 8, 9 and 15a-d).
In figure 8 in the results section there was already a decline in total sponge volume before spring break (3/4/16). If this is a result of feeding it is possibly due to too low a dosage throughout the span of the study and a slow starvation that was exacerbated by complete absence of food over the spring break period. Unfortunately there is no way to know for sure what caused this decrease in volume in this particular trial.

For this project in particular there are many directions to pursue in order to flesh out the best method for keeping sponges in captivity. Several studies have found that a variety of sponge species filter a specific variety of groups from the water column (Strimaitis 2012 and Osinga et al. 1999c). Additionally, other authors suggest that a multispecific diet is preferable for sponge health (Issacs et al. 2009, Mohamed et al. 2008). To put those observations to work in this setting, one would test which bacteria, picoplankton ect. *H. corrugata* filters out of the water column, then culture each of those species in the lab and feed that concoction to *H. corrugata* daily.

The effects of using live rock need to be directly tested. Live rock has been observed to have a positive impact on coral growth in captivity (Yuen 2009). This is an important avenue to pursue, especially because it has not been used nor quantified in a closed system for sponge cultivation in the past.
Another obvious avenue would be to investigate the success of other species in this system, alone and together. Finally, despite my best efforts, several of the sponges in the growth comparison experiment brought “hitchhikers” with them, in the forms of colonial tunicates, anemones and hydroids. So far it is inconclusive how these hitchhikers affect sponge growth. Over the winter break period for the pilot work several anemones and hydroids survived, despite the demise of their host. Work by Swain and Wulff (2007) posits that sponge-zoanthid interactions are generally mutualisms — they go on to note that these relationships can actually inform higher order systematics. Since sponge-zoanthid symbioses are a common feature of Caribbean sponges it is possible that this a feature of sponges in the North Florida region. This could indicate how essential having these symbionts is for in-tank sponge survival.

In conclusion, this system is a good base model for keeping sponges in a laboratory setting. A protein skimmer, regular water flow, live rock and sufficient food are key pieces for an ecologically relevant system in which to keep sponges for further experimentation. There are still several issues to work out, such as how much and what to feed sponges and the effects of hitchhiking organisms. Despite these gaps in our knowledge the sponges are still alive, albeit not exactly healthy. With the goal of filling these gaps, new steps can be taken to confirm how to most effectively keep sponges in captivity.
Chapter 2: Some Natural History About *Halichondria corrugata*

**Introduction**

Many species have specific characteristics that lend them to being excellent model organisms for a particular question. Species-specific traits are essential considerations when selecting a question and when assessing the results of an experiment. Very little information is known about the distribution and ecology of *Halichondria corrugata*. *H. corrugata* was described in 1993 and no studies outside of the Wulff lab have focused on this species since its official description. In this region of Florida it was previously referred to as *Halichondria panicea* (Diaz et al. 1993). Using the ideas from similar species helps us look for aspects of interest of *H. corrugata*’s biology specifically. Below we will give a brief overview of the distribution and ecology of this species.

**Ecology of the genus *Halichondria***

The genus *Halichondria*, Fleming, 1828 is characterized by having a spicule skeleton comprised of only oxea and a rather confused skeletal structure where the organization of the outer layer is different from the endosome (Hooper et al 2002). Members of the genus *Halichondria* are: cosmopolitan in distribution (Diaz et al. 1993), abundant in several habitats across many studies (Wulff 2009, Peattie et al. 1981), and serve as a habitat for a variety of microorganisms (Ávila 2014). They are thought to be highly palatable to species across phyla (Wulff 2009, Kaiser 2016).

Members of the genus *Halichondria* can be preyed upon by other organisms in their respective environments which will affect the ecology and distribution of *Halichondria*. Overall sponge volume and number of individuals of a different species of *Halichondria* were seen to decline between years in Belize with the appearance of French angelfish at the site (Wulff 2009). That being said, no bite marks nor evidence of feeding were recorded on any of the *H. corrugata* individuals at the dock (nor, thankfully, in the lab).

In trials where *Echinaster spp.* starfish were given choices of different sponges various *Halichondria* species were consumed anywhere from 0 to 100% of the time, with each tested species of *Halichondria* falling in each class (consumed 0%, 44% and 100% of the time respectively) (Kaiser 2016).

Several species of *Halichondria* have been recorded as hosts for a myriad of endosymbionts. Peattie et al. (1981) found that certain symbiotic species associated themselves with *Halichondria panicea* as a function of depth and perhaps, current. Sponges’ morphology plays a role in inquiline communities. Individuals of *H. melanadocia* with more dynamic morphologies (fistulose, spherical ect.) had fewer faunal organisms than those with simpler growth forms (Ávila et al 2014). *H. melanadocia* is also known to grow at the base of *Thalassia testudinum* shoots to the benefit of both species, provided there is a medium density of sea grass (Archer et al. 2015). Although the sponge partially shades the sea grass, the sea grass benefits from nutrients released by the sponge. *H. phakelloides* harbors bacteria that have an unusually high concentration of molybdenum (Buccella et al. 2014). The coloration of, *H. panicea* is thought to be due to microbial or bacterial symbionts (Hooper and VanSoest 2002), which could mean the occurrence of symbionts on *H. corrugata* is likely.
This background knowledge, coupled with some fascinating observations from the tank experiments have led us to investigate drivers of coloration and growth form in *H. corrugata* that we did not know before.

**Methods**

**Sponge Identification**

*H. corrugata* was identified by using the methods described in Boury-Esnault and Rützler (1997). A piece of sponge tissue was removed with a razor blade and was dissolved in bleach for three days. The sample was then centrifuged and rinsed with bleach two more times, then centrifuged again in between three water rinses. Finally, most of the water was pipetted out and the spicules are poured onto a labeled microscope slide and then set on a slide warmer to dry. Once the spicules were dry, a mounting medium was added to the slide and covered with a cover slip. The slide was examined and spicule types were drawn and measured. Spicule size ranges were compared to those found in the literature, along with external characteristics like morphology and color (Boury-Esnault, and Rützler, 1997). The identification of *Halichondria corrugata* for this study came from Diaz et al. 1993 and from the ID expertise of Kathleen Kaiser from the Wulff lab.

**Symbiont Identification**

As time went on, the pieces of *H. corrugata* in the large tank system became noticeably lighter in color. From a dark green nearly black to a light olive green, sometimes with yellow edges on fistulose morphologies. It seemed clear something was changing, especially since sponges with this coloration had not been seen at the dock site. Slices of live *H. corrugata* tissue were taken from the individuals in the 9.46L tank (which was outfitted with only a pump) at the start of the large tank experiment and then again at 68 days. Slices were made with a razor blade and were thin enough that light could easily shine through the tissue. The slice was then quickly transferred onto a microscope slide,

*Figs 1 and 2*

These two photos represent the same sponge at t= 7 and t= 58. The morphology and coloration are drastically different. The sponge also decreased in volume over this time period.
covered with a cover slip and observed immediately under the microscope. Photos of the
magnified sponge were taken at 400x, 600x and 1000x magnification. The 1000x
magnification utilized oil immersion.

These photos were then analyzed using the software ImageJ. Small dots were apparent
between the spicules and mesohyle of the sponge. The number of pixels comprising the
diameter of one of these dot was analyzed. 20 dots in each photo were measured. This
diameter in pixels was then converted into a measurement using the scale in the
microscope objective and a calibrated pixel to unit value that came from taking the number
of pixels between the tick marks on the objective. This number was then multiplied by the
conversion factor for the specific magnification. This gave a size of the dots in µm. These
sizes were then compared to information in the literature about microbial symbionts found
in sponges.

Flow Rate Analysis

Throughout the course of the experiment the sponge morphology changed from
rugose to fistulose (Boury-Esnault and Rützler 1997). In many cases the ends of the
fistulose bits of sponge were lighter green or yellow than the rest of the sponge (figs 1 and
2). The dock site appeared to have some different morphologies in different locations on
the dock. A transect was run along both edges of the dock and photos were taken every
1.52m. Flow rate was measured by dropping 5ml of canned coconut milk into the water
underneath a checkered plastic sheet. The checkered sheet had a grid of 2.54cm squares,
which allowed us to see if the flow also moved at an angle relative to the dock. The amount
of time the leading edge of the stream of coconut milk took to flow from the initial point to
the edge of the plastic bag was recorded.
Results

Symbiont Identification

The average size of the small dots in the images ranged from 1.41-3.26μm in diameter. They were frequently found clustered together, especially along spicules (figs 3-5).

Fig 3. 1000x magnification using oil immersion. Note dots clustered around edge of spicule.
Flow rate analysis
At the dock site sponge morphology did not appear to change along either the land-side or canal-side of the dock.

<table>
<thead>
<tr>
<th>Site</th>
<th>Average Flow rate (cm/s)</th>
<th>Example Sponge 15m along transect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Land-side (n=3)</td>
<td>3.437</td>
<td></td>
</tr>
</tbody>
</table>
Discussion

Symbiont identification

None of the dots or speckles look like structures I have seen in a typical sponge semithin cross section, especially after examining "The Cell Biology of Sponges" (Simpson 1984). That, coupled with recorded diameter ranges for photosynthetic microbial symbionts, would suggest that *Halichondria corrugata* has photosynthetic microbial symbionts. Freeman and Thacker (2011) note that photosynthetic microbial symbionts generally fall in the 1-2µm diameter size class. Cyanobacteria can range in diameter from 1.0 to 5.0 µm and 6.0-12.0µm (Simpson 1984). Remember that the sponges in the experimental tank changed color from dark black/green to a light olive green. The grow light on the tank was added quite late in the process (at day 48), so it is possible the coloration change was due to the photosynthetic microbial symbionts leaving the sponge in the absence of a direct light source. Simpson (1984) referenced a study by Liaci (1964b) where they found that the quantity of pigment in a sponge, coupled with symbiont density are together directly related to the amount of available sunlight (fig 6). This observation supports the idea that microsymbionts are driving coloration in *Halichondria corrugata*. At the dock site, dark green individuals of *H. corrugata* and flattened tops along the edge of the dock, this could be due to growing up right against the water line, or that they are trying to optimize light reception for their symbionts (Taylor et al. 2007). As noted in Chapter One, *H. corrugata* was seen to host both anemones and hydroids. Zoanthids have been observed to occur more frequently in sponges with photoendosymbionts, (especially in zoanthids with photoendosymbionts) (Swain and Wulff 2007). Although it was not tested here, by counting the number of symbionts present in pale and dark colored sponges we could see if the symbionts do, in fact play a role in pigmentation. If pigment could be correlated with symbiont density for certain species field assessments of organism ecology would become that much easier.
Flow rate analysis
The sample size of the flow rate experiments is too low to draw any meaningful conclusions but it does hint at various areas to focus on in the future. The dock simply floated on Styrofoam rather than on a network of pilings so the dock’s effect on flow would be minimal compared to the effect from traditional docks. The morphologies of *H. corrugata* individuals on either side of the dock, at matched distances along a transect were not notably different. Perhaps flow was not the driving force for the drastic change in morphology that was seen in the experimental tanks from Chapter One. This trend toward finger-like projections, however, has been observed in populations of coral reef sponges that were transplanted onto mangrove roots, where there is less flow than on a coral reef (Wulff, personal communication). Palumbi (1984) posited that stress also jump-starts morphological change in plastic species over a period of several months. The author of this study looked at mechanical stress, in the form of wave action, on morphology and found that *Halichondria panicea* was quicker to change its morphology when exposed to increased stress rather than when exposed to decreased stress. Perhaps the morphology change seen in Chapter One was due to starvation rather than flow or mechanical stress. This has been posited in Duckworth et al. (2005). That being said, both the sponges in the larger tank system (with regular feeding) and the small 9.46L system (with biweekly feeding) exhibited the same pale, fistulose morphology. Given that the same effect was seen in tanks with different feeding regimes I posit that flow might still be a contributing factor. The benefit of having noted the drastic morphology changes in a tank system makes it easy to test. That these morphology changes have been noted in the field make it a worthy and ecologically relevant endeavor.

**fig. 6**
Note difference in coloration of two different *H. corrugata* individuals at the dock. Possibly indicative of variable symbiont densities, at different levels of light exposure at the dock site.
Conclusions

As a result of this honor’s thesis we have a much better idea about how to keep sponges alive in captivity. We know that both the protein skimmer and flow in the tank are essential. These facts show us how tanks can become a viable option for long-term experimentation on sponges in the lab. Given the decline we have seen in sponge volume through the course of this experiment with the use of a monospecific food source sponges, likely, need more food with more variety (Osinga et al. 2001) than provided to them in this study. And, finally, *Halichondria corrugata*’s growth is likely affected by flow regimes in the tank and likely harbors photosynthetic microbial symbionts. Despite all of the set backs and the decline in sponge volume, the sponges are still alive in both the main and scaled down tank set-ups. These data about growth rates mean little without field comparisons, especially since we know so little about *Halichondria corrugata* in the first place. Thanks to the lab and field observations recorded as a result of this project we know more about *H. corrugata* now than we did before, and the Wulff lab has lasting infrastructure for future experimentation. We have incorporated effective hobbyist techniques from the aquarist community, like the use of live rock and protein skimmers, into experimental designs in a way that is quantifiable. Finally, we have starting point from which we can establish an effective, and ecologically relevant way, to keep sponges in captivity.
Bibliography


“Comparison of the bacterial communities of wild and captive sponge *Clathria prolifera* from the Chesapeake Bay.” *Marine Biotechnology.* **11:**758-770

Kaiser, K. 2016. “Species distributions and ecological interactions in Apalachee Bay.” (Honors Thesis). Department of Biological Science, Florida State University, Tallahassee, FL.


